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**Riber, Ulla; Hvass, Henriette Cordes; Heegaard, Peter M. H.; Ståhl, Marie ; Jensen, Tim Kåre; Jungersen, Gregers**

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## Detailing profiles of *Lawsonia intracellularis* specific lymphocytes in the immune response to a challenge inoculation after oral vaccination or primary inoculation with virulent bacteria

U. Riber, H. Cordes, P.M.H. Heegaard, M. Ståhl, T.K. Jensen, G. Jungersen

National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1870 Frederiksberg C, Denmark

Vaccination against the intracellular porcine enteric pathogen *Lawsonia intracellularis* remains a challenge as the commercially available vaccine does not provide full protection. In an experimental challenge study, the Enterisol® Ileitis attenuated live vaccine against *Lawsonia intracellularis* did not induce measurable primary humoral or cell-mediated immune responses, nor was it able to reduce faecal shedding of bacteria from eight vaccinated pigs compared to seven age-matched naïve challenge-control pigs. Vaccinated pigs did, however, respond to vaccination with an acute phase protein response in serum comparable to that of ten primary infected (and later re-inoculated) pigs, and post mortem immunohistochemical examination of tissue samples revealed less *L. intracellularis* in vaccinated pigs compared to challenge-control pigs.

In more detailed studies of the immune response after challenge, the vaccinated pigs did not show any immediate evidence of primed (faster or stronger) IgG or CMI response compared to naïve pigs. *L. intracellularis*-specific CMI responses 18-33 dpi were further characterized by flow cytometry for intracellular IFN-γ and cell proliferation (CFSE). Phenotypes of IFN-γ producing cells in the vaccinated pigs showed profiles primarily of $CD8^{+}(CD4^{neg})$ and $CD4^{+}CD8^{+}$ double positive lymphocytes. Similar profiles of IFN-γ producing cells were found in re-inoculated immune pigs, which experienced a boost in CMI responses. Cellular proliferation was identified in nearly all vaccinated pigs with mainly $CD4^{+}(CD8^{neg})$ and $CD4^{+}CD8^{+}$ double positive cells, whereas the immune re-inoculated pigs also included response in $CD8^{high}(CD4^{neg})$ cells. These different profiles of responsive cellular phenotypes may influence the observed differences in protection between vaccinated and re-inoculated pigs.